

## Isolation of viral haemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St Clair, Michigan, USA reveals a new sublineage of the North American genotype

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### Abstract

Viral haemorrhagic septicaemia virus (VHSV) was isolated from muskellunge, *Esox masquinongy* (Mitchill), caught from the NW portion of Lake St Clair, Michigan, USA in 2003. Affected fish exhibited congestion of internal organs; the inner wall of the swim bladder was thickened and contained numerous budding, fluid-filled vesicles. A virus was isolated using fish cell lines inoculated with a homogenate of kidney and spleen tissues from affected fish. Focal areas of cell rounding and granulation appeared as early as 24 h post-inoculation and expanded rapidly to destroy the entire cell sheet by 96 h. Electron microscopy revealed virions that were 170–180 nm in length by 60–70 nm in width having a bullet-shaped morphology typical of rhabdoviruses. The virus was confirmed as VHSV by reverse transcriptase-polymerase chain reaction. Sequence analysis of the entire nucleoprotein and glycoprotein genes revealed the virus was a member of the North American genotype of VHSV; however, the isolate was sufficiently distinct to be considered a separate sublineage, suggesting its origin may have been from marine species inhabiting the eastern coastal areas of the USA or Canada.

**Keywords:** genotype, Great Lakes, muskellunge, pathology, rhabdovirus, viral haemorrhagic septicaemia.

### Introduction

The Great Lakes basin is one of the largest freshwater systems in the world and is known for its rich biodiversity (Coon 1999). Despite its ecological and economic importance, there is limited knowledge about viral infections of fish in the Great Lakes. Over the last two decades, a few fish viruses have been reported from the Great Lakes basin including infectious pancreatic necrosis virus (Birnaviridae), dermal sarcoma virus of walleye (Retroviridae), largemouth bass virus (Iridoviridae), lymphocystis virus (Iridoviridae) and epizootic epitheliotropic disease virus (Herpesviridae), as reviewed by Faisal & Hnath (2005).

Lake St Clair is a major lake in the Great Lakes system that has historically supported an economically and socially important sport fishery for smallmouth bass, *Micropterus dolomieu* (Lacepède); walleye, *Sander vitreus vitreus* (Mitchill); yellow perch, *Perca flavescens* (Mitchill); and muskellunge, *Esox masquinongy* (Mitchill) (Haas 1978). For example, muskellunge abundance tripled during the 1980–90s as water clarity increased, and macrophyte densities and spatial coverage increased. The muskellunge population in Lake St Clair is completely self-sustaining (Bryant & Smith 1988), thus any threat to the continued successful recruitment of muskellunge in the lake is a serious concern for fishery managers.

Over the last 3 years, a survey of Lake St Clair's muskellunge was conducted to determine the spread of *Piscirickettsia* sp., an intracellular bacter-

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ium associated with granulomatous skin lesions in adult muskellunge in this lake. During the course of this survey, a rhabdovirus was isolated from four of 42 muskellunge examined. No other rhabdoviruses have been reported from Great Lakes fish species. In North America, only four rhabdoviruses are known to infect fish, namely; infectious haematopoietic necrosis virus, viral haemorrhagic septicaemia virus (VHSV), spring viraemia of carp virus and starry flounder rhabdovirus (Mork, Hershberger, Kocan, Batts & Winton 2004). This study was initiated to identify the relatedness of the new isolate from muskellunge to other fish rhabdoviruses.

## Materials and methods

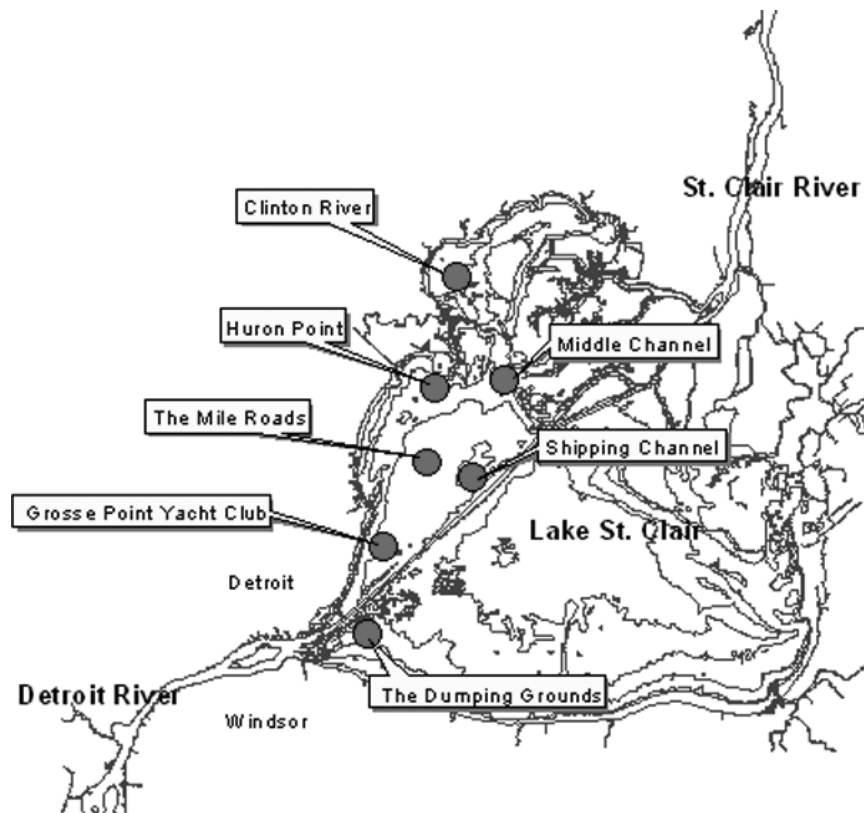
### Fish

During 2003–2005, a total of 42 muskellunge, ranging in weight from 4.0 to 14.2 kg, were collected from seven locations in the NE portion of Lake St Clair by personnel from the Michigan Department of Natural Resources stationed at the

Mt Clemens Research Station (Fig. 1). Fish were transported alive to Michigan State University Aquatic Animal Health Laboratory, where they were processed immediately and samples were taken from internal organs under aseptic conditions.

### Virus isolation

Virus isolation was performed according to the standard protocols published by the American Fisheries Society (2004) and the Office International des Epizooties (2003). Samples from kidneys, spleen and swim bladder lesions were excised aseptically from individual fish, weighed, diluted with nine volumes of antibiotic-free minimum essential medium (MEM; Sigma Chemical Co., St Louis, MO, USA), and then homogenized using a Biomaster Stomacher-80 (Wolf Laboratories Ltd, Pocklington, York, UK) at the high-speed setting for 2 min. Homogenates were allowed to settle, while kept on ice, for 15 min and an aliquot of cell culture medium was added to produce  $10^{-2}$  and



**Figure 1** Sites of trap nets at Lake St Clair that were used to collect muskellunge used in this study.

$10^{-3}$  dilutions (w/v) of the original tissues. For the initial isolation, the fathead minnow (FHM) and the chinook salmon embryo (CHSE-214) cell lines were used. Tissue culture flasks were inoculated with dilutions of the homogenate (two flasks/dilution). Inoculated cells were incubated at 20 and 15 °C for FHM and CHSE-214, respectively, and examined for the appearance of cytopathic effects (CPE) for 21–28 days post-inoculation. Homogenates were also prepared from skin lesions and treated as described above. Growth of viral isolates and additional studies were performed using FHM and the 'Epithelioma papulosum cyprini' (EPC) cell line grown at 25 °C. Medium from cell cultures showing CPE was stored in aliquots at –80 °C for use as stock virus.

### Electron microscopy

Medium from cell cultures showing CPE was replaced with 5 mL of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). Following fixation for 2 h at 4 °C, cells were harvested and centrifuged at 1500 *g* for 10 min; then, the cell pellet was collected, post-fixed in 1% osmium tetroxide for 90 min, and embedded in epoxy resin according to the protocols of Chang, Pan, Wu & Kuo (2002). Ultrathin sections were examined with a JEOL JEM-100 electron microscope (JEOL USA, Peabody, MA, USA).

### Virus identification by polymerase chain reaction

Six-day-old EPC cells in 25 cm<sup>2</sup> flasks were infected with 0.2 mL of the muskellunge isolate.

At 3 days post-infection, when CPE was nearly complete, the remaining cells were scraped from the flask, as were cells from an uninfected control flask. The culture fluids were centrifuged at 200 *g* for 2 min and the pellets resuspended in 1.5 mL of MEM. As polymerase chain reaction (PCR) controls, 0.5 mL of the resuspended pellet from the uninfected EPC culture was transferred into each of two 1.5 mL tubes to which 20 µL of a British Columbia herring isolate of VHSV (positive control) or nothing (negative control) was added. Total RNA was extracted with TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. Pellets of RNA were resuspended in 50 µL of deionized water, heated at 60 °C for 10 min, and cooled on ice until added to reverse transcriptase (RT)-PCR reaction mixtures. Reverse transcription and PCR of the central region of the glycoprotein (G) gene of VHSV was performed following the procedures outlined in Hedrick, Batts, Yun, Traxler, Kaufman & Winton (2003) and Winton & Einer-Jensen (2002). Thirty PCR cycles amplified a 914-bp region using the central G primers (Table 1). In order to amplify the entire G gene (1609 nt) and N gene (1386 nt) of the muskellunge isolate, additional primers were substituted in subsequent RT-PCR reactions to amplify the 5'-G, 3'-G, 5'-N, Central N and 3'-N according to the list of primers in Table 1.

### Sequence analysis

The PCR products were purified with a StrataPrep PCR purification kit (Stratagene, La Jolla, CA, USA) and sequenced with a fluorescent dye termi-

**Table 1** Primers used in this study

Primer code	Primer sequence (5' to 3')	nt	Primer location on 07–71 VHSV (AJ233396)	Expected PCR products (base pairs)
5'-G+	5'-TTAGACATGGGAGTGTGACTT-3'	21	2647–2667	771
5'-G–	5'-CCAGATGCAGGARGGTTTC-3'	18	3417–3400	
Central G+	5'-ACTACCTACACAGAGTGAC-3'	19	3251–3269	914
Central G–	5'-CAATTGTCCCCGAATATCAT-3'	21	4164–4144	
3'-G+	5'-GTGGTCAGCATCACTACAA-3'	20	4045–4064	550
3'-G–	5'-GGAGAGAAGCTGGTTGTGCTG-3'	21	4594–4574	
5'-N+	5'-ATGATGAGTTATGTTACARGG-3'	21	13–33	587
5'-N–	5'-TTGTCCACCGAGTACTTGGT-3'	20	599–580	
Central N+	5'-CTGGAGGCAAGTGCAAG-3'	18	406–423	588
Central N–	5'-GTATCGCTCTTGGATGGAC-3'	19	993–975	
3'-N+	5'-GTTGAGTCAGCCAGRCG-3'	17	922–938	645
3'-N–	5'-CYTTRGGGGCGTTGTCTAG-3'	19	1566–1548	

VHSV, Viral haemorrhagic septicaemia virus; PCR, polymerase chain reaction.

Genotype	Isolate name	Year	Host species	GenBank no.
I	DKF1	1962	Rainbow trout	AF345857
I	DKHeddam	1972	Rainbow trout	Z93412
Ia*	FR0771	1971	Rainbow trout	AY546616
Ia	DK7380	1994	Rainbow trout	AY546594
Ib	DKMrhabdo	1979	Atlantic cod	Z93414
Ib	DK6p403	1999	Atlantic herring	AY546584
Ic	DK2835	1982	Rainbow trout	AY546585
Ic	DK5131	1988	Rainbow trout	AF345858
Id	Flka422	2000	Rainbow trout	AY546615
Id	NOA16368G	1968	Rainbow trout	AY546621
Ie	GE12	1981	Rainbow trout	AY546619
II	DK1p52	1996	Sprat	AY546576
II	DK1p53	1996	Atlantic herring	AY546577
III	DK4p101	1997	Whiting	AY546581
III	FRL59X	1987	Eel	AY546618
III	UK86094	1994	Turbot	AY546628
III	UKMLA986P	1998	Norway pout	AY546632
IVa	USMakah	1988	Coho salmon	U28747
Ib	JP96KRRV9601	1996	Japanese flounder	DQ401190
IVa	JP99Obama25	1999	Japanese flounder	DQ401191
IVa	WA91Clearwater	1991	Coho salmon	DQ401189
IVa	BC99292	1999	Atlantic salmon	DQ401188
IVa	BC98250	1998	Atlantic salmon	DQ401187
IVa	ME03	2003	Atlantic herring	DQ401192
IVa	BC99001	1999	Pacific sardine	DQ401195
IVa	BC99010	1999	Pacific herring	DQ401194
IVa	BC93372	1993	Pacific herring	DQ401186
IVb	MI03GL	2003	Muskellunge	DQ401193

The first 18 isolates were selected from among the 74 isolates analysed by Einer-Jensen *et al.* (2004).

The remaining isolates were sequenced as part of this study and the sequences deposited in GenBank.

\*Sublineages assigned to genotypes I and IV.

**Table 2** Data for the 28 viral haemorrhagic septicaemia virus isolates used in this study for phylogenetic analysis (Fig. 4)

nator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) in individual reactions using the primers listed in Table 1. The full-length G and N gene sequences were submitted to GenBank as nos DQ401193 and DQ427105, respectively. In order to compare the muskellunge sequences with the representatives of the genotypes found in fish of the Pacific coast of North America, Europe and Japan, we trimmed all G gene sequences listed in Table 2 to the 1521 nt open-reading frame of the G protein, and aligned these with ClustalW. Analysis by PAUP4.0-generated neighbour-joining and parsimony phylogenetic trees containing 1000 bootstrap samplings of the data. Genotype II was selected as an outgroup for both analyses and branches were collapsed for confidence values below 70%.

## Results and discussion

In the four virus-infected fish, there was severe congestion of kidney, spleen, liver and gastrointestinal tract. Fluid-filled vesicles budded from the internal membrane of the swimbladder (Fig. 2). It should be noted that a bacterial pathogen, *Piscirickettsia* sp., has also been associated with raised,



**Figure 2** Inner membrane of the swim bladder of an adult, infected muskellunge caught from Lake St Clair. Note the thickening of the membrane and the budding fluid-filled vesicles.

haemorrhagic lesions in Lake St Clair muskellunge, however, none of the four fish from which the virus was isolated exhibited these characteristic lesions.

Cytopathic effect appeared in the FHM and CHSE-214 cultures inoculated with kidney/spleen homogenates from four of 27 (14.8%) fish examined. No CPE was observed in cells inoculated with homogenates of skin tissues exhibiting the external lesions. CPE was more prominent in FHM than

CHSE-214 cultures and was in the form of focal areas of rounded, refractile cells that progressed to full lysis of the cell monolayer within 4 days post-inoculation.

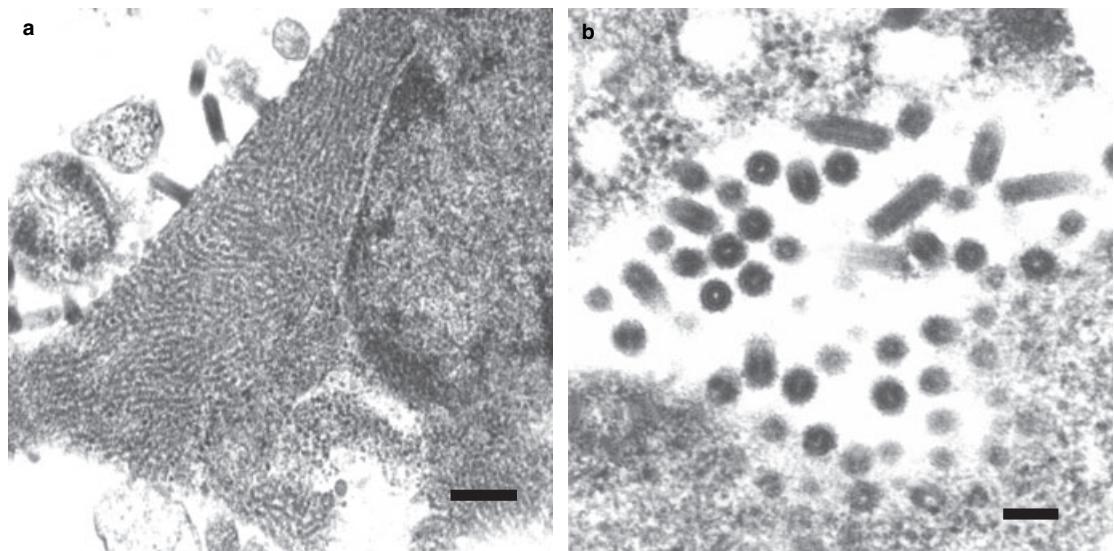
Electron microscopy of infected cell lines revealed bullet-shaped viral particles having the characteristic morphology of members of the family Rhabdoviridae (Fig. 3a,b). Many virus particles were present in the intra- and extracellular spaces. The virions appeared to possess an envelope around a striated nucleocapsid. Enveloped virions measured 170–180 nm in length by 60–70 nm in width.

The muskellunge virus isolate was identified as VHSV by RT-PCR. Initially, VHSV was known for its ability to produce high mortality rates in rainbow trout, *Oncorhynchus mykiss* (Walbaum), and other salmonids in Europe (Wolf 1988; Smail 1999), although the virus was also isolated from non-salmonids (Meier, Schmitt & Wahli 1994). Over the past two decades, VHSV has been recovered from an increasing variety of marine fish collected from the coastal waters of North America (Meyers, Sullivan, Emmenegger, Follett, Short, Batts & Winton 1992; Meyers & Winton 1995; Hedrick *et al.* 2003), Europe (Dixon, Feist, Kehoe, Parry, Stone & Way 1997; Mortensen, Heuer, Lorenzen, Otte & Olesen 1999; King, Snow, Smail & Raynard 2001; Brudeseth & Evensen 2002) and Asia (Takano, Mori, Nishizawa, Arimoto &

Muroga 2001; Kim, Lee, Hong, Park & Park 2003).

In some instances, VHSV was associated with lesions or mortality (Schlotfeldt, Ahne, Vestergaard Jørgensen & Glende 1991; Meyers, Short, Lipson, Batts, Winton, Wilcock & Brown 1994; Ross, McCarthy, Huntly, Wood, Stuart, Rough, Smail & Bruno 1994; Marty, Freiberg, Meyers, Wilcock, Farver & Hinton 1998; Dixon 1999; Meyers, Short & Lipson 1999; Smail 2000; Skall, Olesen & Møllergaard 2005). In other cases, the virus has been isolated from apparently healthy fish which were believed to act as virus reservoirs (Kent, Traxler, Kieser, Richard, Dawe, Shaw, Prosperi-Porta, Ketcheson & Evelyn 1998; Takano, Nishizawa, Arimoto & Muroga 2000; Dopazo, Bandín, López-Vasquez, Lamas, Noya & Barja 2002).

Isolates of VHSV have been analysed at the sequence level and four genotypes of the virus appear to be distributed geographically, rather than by host or year of isolation (Benmansour, Basurco, Monnier, Vende, Winton & de Kinkelin 1997; Stone, Way & Dixon 1997; Snow, Cunningham, Melvin & Kurath 1999; Nishizawa, Iida, Takano, Isshiki, Nakajima & Muroga 2002; Thiéry, de Boissésion, Jeffroy, Castric, de Kinkelin & Benmansour 2002; Kim *et al.* 2003; Einer-Jensen, Ahrens, Forsberg & Lorenzen 2004; Einer-Jensen, Ahrens & Lorenzen 2005a; Snow, Bain, Black,

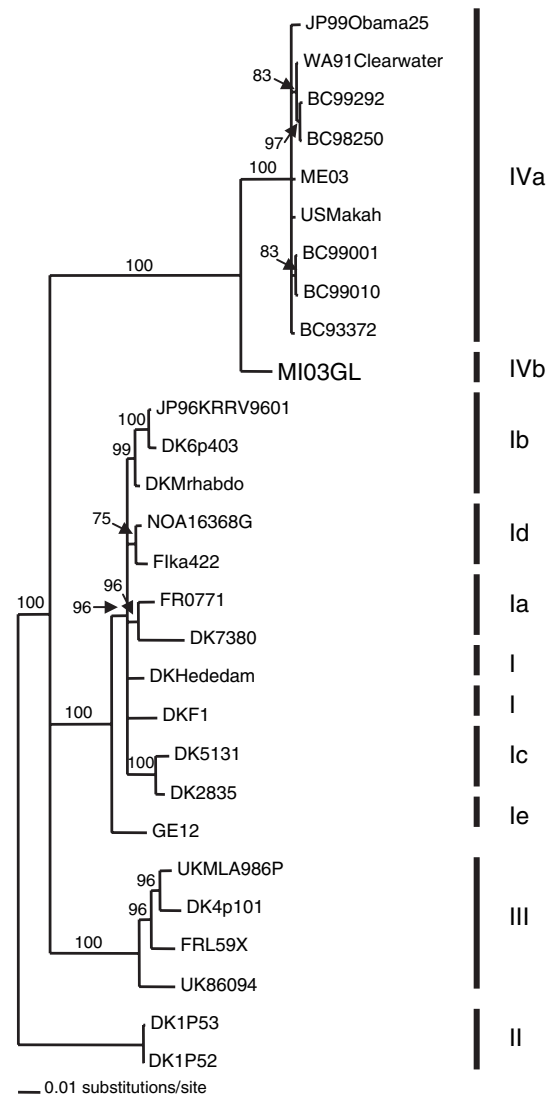


**Figure 3** Electron micrographs of the rhabdovirus isolated from Lake St Clair muskellunge. (a) Fathead minnow cells infected for 72 h with the muskellunge isolate. Notice the mature bullet-shaped virions at the cell surface (bar = 200 nm). (b) Higher magnification cross and longitudinal sections of bullet-shaped virions (bar = 100 nm).

Taupin, Cunningham, King, Skall & Raynard 2005). Genotypes I, II and III are mainly found in Europe and Japan, while isolates of genotype IV have, to date, only been recovered from fish in North America, Japan and Korea (Einer-Jensen, Winton & Lorenzen 2005b; Snow *et al.* 2005).

Nucleotide sequence analysis of the glycoprotein gene demonstrated that the 2003 virus isolate from muskellunge was most closely related to the North American genotype IV of VHSV and was clearly distinct from the three European genotypes (Fig. 4). This was not entirely surprising as Hedrick *et al.* (2003) demonstrated that the North American genotype of VHSV has a wide host and geographical range that includes many marine species inhabiting the Pacific coastal areas of the USA and Canada such as Pacific sardine, *Sardinops sagax* (Jenyns), from coastal waters of Vancouver Island, British Columbia, Canada, eulachon, *Thaleichthys pacificus* (Richardson), and surf smelt, *Hypomesus pretiosus* (Girard), from Oregon and Pacific mackerel, *Scomber japonicus* (Houttuyn), from southern California. However, sequence comparisons of genotype IV isolates from North America that have been analysed until now, have tended to show a very low level of genetic diversity (0.2–0.6%), perhaps reflecting an older, well-established host–pathogen relationship. The VHSV isolate from muskellunge reported here differed from other genotype IV isolates by 3.6–3.7% at the nucleotide level, and neighbour-joining analysis placed the isolate in a distinct sublineage (IVb) of the phylogenetic tree (Fig. 4). Parsimony analysis also confirmed this separation (data not shown).

The sequence difference between this 2003 isolate from the Great Lakes and typical isolates of VHSV from the Pacific coastal areas of North America, Japan or Korea suggests the isolate from Great Lakes muskellunge was not directly introduced from either Europe, Asia or from the Pacific coast of North America, but is probably endemic among marine fish from the Atlantic coast of North America. However, an isolate of VHSV recovered from Atlantic herring, *Clupea harengus harengus* L., collected in marine waters near the state of Maine in 2003 had a sequence nearly identical to isolates from the West coast of North America (isolate ME03 in Fig. 4). To further increase the level of uncertainty, VHSV was isolated from 50% (15 of 30) of the Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), captured in July 1994 at the



**Figure 4** Phylogenetic distance tree generated by neighbour-joining analysis of 28 complete glycoprotein gene open-reading frame sequences (1521 nt) representing the four major viral haemorrhagic septicaemia virus (VHSV) genotypes. Genotypes and sublineages are depicted by bold vertical lines as described by Einer-Jensen *et al.* (2004). Inclusion of the muskellunge isolate from Michigan expands the North American VHSV genotype into sublineages IVa and IVb. Virus isolate codes are described in Table 2 and bootstrap confidence values are shown at branch nodes.

Flemish Cap near the Atlantic coast of Canada (Dopazo *et al.* 2002) and determined to belong to genotype III (Einer-Jensen *et al.* 2005b). Olivier (2002) reported the isolation of a North American strain of VHSV from a large outbreak in mummichog, *Fundulus heteroclitus* (L.), in New Brunswick, Canada, but the sequence of this isolate is not yet published.

The emergence of VHSV in the Great Lakes does not seem to be a single event. During the spring/summer of 2005, a large fish kill occurred in eastern Lake Ontario, Canada among freshwater drum, *Aplodinotus grunniens* (Rafinesque), and other species including muskellunge, goby, *Neogobius melanostomus* (Pallas), and an unidentified species of bass (Canadian Cooperative Wildlife Health Centre 2005). In the spring of 2006, large mortalities were recorded among several additional species of fish in Lake St Clair, Lake Erie and Lake Ontario and VHSV was isolated from both moribund and normal-appearing fish. The isolates of VHSV from Great Lakes fish that have been examined to date were essentially identical to the isolate reported here. The historic absence of VHSV in past health surveys and the recovery of identical isolates of VHSV from large numbers of dying fish in several of the Great Lakes suggest that the virus may have been recently introduced into the Great Lakes through one of several potential sources including ballast water or by anadromous or catadromous species that can enter the Great Lakes via the St Lawrence river.

As the broad-host range of VHSV is known to include several important Great Lakes fish species including northern pike, *Esox lucius* L.; lake trout, *Salvelinus namaycush* (Walbaum); rainbow trout, brook trout, *Salvelinus fontinalis* (Mitchill); brown trout, *Salmo trutta* L.; and Arctic grayling, *Thymallus arcticus* (Pallas), the virus may represent a greater threat to the fisheries in the region than is currently understood. At present, VHSV appears to have become established among several species in the Great Lakes which can serve as reservoirs to maintain the virus for future outbreaks, and efforts should be made to prevent further extension of the geographical range into other important freshwater systems.

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### References

- American Fisheries Society (2004) *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens*. American Fisheries Society, Bethesda, MD.
- Benmansour A., Basurco B., Monnier A.F., Vende P., Winton J.R. & de Kinkelin P. (1997) Sequence variation of the glycoprotein gene identifies three distinct lineages within field isolates of viral haemorrhagic septicaemia virus, a fish rhabdovirus. *Journal of General Virology* **78**, 2837–2846.
- Brudeseth B.E. & Evensen O. (2002) Occurrence of viral haemorrhagic septicaemia virus (VHSV) in wild marine fish species in the coastal regions of Norway. *Diseases of Aquatic Organisms* **52**, 21–28.
- Bryant W.C. & Smith K.D. (1988) *The Status of the Muskellunge in Lake St Clair, Michigan, 1978–86*. Michigan Department of Natural Resources, Fisheries Research Report 1955, Lansing, MI.
- Canadian Cooperative Wildlife Health Centre (2005) A mortality event in freshwater drum (*Aplodinotus grunniens*) from Lake Ontario, associated with viral hemorrhagic septicemia virus (VHSV), type IV. *Wildlife Health Centre Newsletter* **11**, 10.
- Chang P.H., Pan Y.H., Wu C.M. & Kuo S.T. (2002) Isolation and molecular characterization of herpesvirus from cultured European eels *Anguilla anguilla* in Taiwan. *Diseases of Aquatic Organisms* **50**, 111–118.
- Coon T.J. (1999) Ichthyofauna of the Great Lakes Basin. In: *Great Lakes Policy and Management* (ed. by W.W. Taylor & C.P. Ferreri), pp. 55–71. Michigan State University Press, East Lansing, MI.
- Dixon P.F. (1999) VHSV came from the marine environment: clues from the literature, or just red herrings? *Bulletin of the European Association of Fish Pathologists* **19**, 60–65.
- Dixon P.F., Feist S., Kehoe E., Parry L., Stone D.M. & Way K. (1997) Isolation of viral haemorrhagic septicaemia virus from Atlantic herring *Clupea harengus* from the English Channel. *Diseases of Aquatic Organisms* **30**, 81–89.
- Dopazo C.P., Bandín I., López-Vasquez C., Lamas J., Noya M. & Barja J.L. (2002) Isolation of viral hemorrhagic septicaemia virus from Greenland halibut *Reinhardtius hippoglossoides* caught at the Flemish Cap. *Diseases of Aquatic Organisms* **50**, 171–179.
- Einer-Jensen K., Ahrens P., Forsberg R. & Lorenzen N. (2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology* **85**, 1167–1179.
- Einer-Jensen K., Ahrens P. & Lorenzen N. (2005a) Parallel phylogenetic analyses using the N, G or Nv gene from a fixed group of VHSV isolates reveal the same overall genetic typing. *Diseases of Aquatic Organisms* **67**, 39–45.
- Einer-Jensen K., Winton J. & Lorenzen N. (2005b) Genotyping of the fish rhabdovirus, viral haemorrhagic septicaemia virus, by restriction fragment length polymorphisms. *Veterinary Microbiology* **106**, 167–178.
- Faisal M. & Hnath J.G. (2005) Fish health and disease issues in the Laurentian Great Lakes. In: *Health and Diseases of Aquatic Organisms: Bilateral Perspectives* (ed. by R.C. Cipriano, I.S. Shchelkunov & M. Faisal), pp. 331–349. Michigan State University, East Lansing, MI.
- Haas R.C. (1978) The muskellunge of Lake St. Clair. *American Fisheries Society Special Publication* **11**, 334–339.

- Hedrick R.P., Batts W.N., Yun S., Traxler G.S., Kaufman J. & Winton J.R. (2003) Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. *Diseases of Aquatic Organisms* **55**, 211–220.
- Kent M.L., Traxler G.S., Kieser D., Richard J., Dawe S.C., Shaw R.W., Prosperi-Porta G., Ketcheson J. & Evelyn T.P.T. (1998) Survey of salmonid pathogens in ocean-caught fishes in British Columbia, Canada. *Journal of Aquatic Animal Health* **10**, 211–219.
- Kim S.M., Lee J.I., Hong M.J., Park H.S. & Park S.I. (2003) Genetic relationship to the VHSV (viral hemorrhagic septicemia virus) isolates from cultured olive flounder, *Paralichthys olivaceus*, in Korea. *Journal of Fish Pathology* **16**, 1–12.
- King J.A., Snow M., Smail D.A. & Raynard R.S. (2001) Distribution of viral haemorrhagic septicaemia virus in wild fish species of the North Sea, North East Atlantic Ocean and Irish Sea. *Diseases of Aquatic Organisms* **47**, 81–86.
- Marty G.D., Freiberg E.F., Meyers T.R., Wilcock J., Farver T.B. & Hinton D.E. (1998) Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring (*Clupea pallasii*) spawning in Prince William Sound, Alaska, USA. *Diseases of Aquatic Organisms* **32**, 15–40.
- Meier W., Schmitt M. & Wahli T. (1994) Viral hemorrhagic septicemia (VHS) of nonsalmonids. *Annual Review of Fish Diseases* **4**, 359–373.
- Meyers T.R. & Winton J.R. (1995) Viral hemorrhagic septicemia virus in North America. *Annual Review of Fish Diseases* **5**, 3–24.
- Meyers T.R., Sullivan J., Emmenegger E., Follett J., Short S., Batts W.N. & Winton J.R. (1992) Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska, USA. *Diseases of Aquatic Organisms* **12**, 167–175.
- Meyers T.R., Short S., Lipson K., Batts W.N., Winton J.R., Wilcock J. & Brown E. (1994) Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasii* from Prince William Sound and Kodiak Island, Alaska, USA. *Diseases of Aquatic Organisms* **19**, 27–37.
- Meyers T.R., Short S. & Lipson K. (1999) Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fish. *Diseases of Aquatic Organisms* **38**, 81–86.
- Mork C., Hershberger P., Kocan R., Batts W. & Winton J. (2004) Isolation and characterization of a rhabdovirus from starry flounder (*Platichthys stellatus*) collected from the northern portion of Puget Sound, Washington, USA. *Journal of General Virology* **85**, 495–505.
- Mortensen H.F., Heuer O.E., Lorenzen N., Otte L. & Olesen N.J. (1999) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. *Virus Research* **63**, 95–106.
- Nishizawa T., Iida H., Takano R., Isshiki T., Nakajima K. & Muroga K. (2002) Genetic relatedness among Japanese, American and European isolates of viral hemorrhagic septicemia virus (VHSV) based on partial G and P genes. *Diseases of Aquatic Organisms* **48**, 143–148.
- Office International des Epizooties (2003) *Manual of Diagnostic Tests for Aquatic Animals*, 4th edn. Office International des Epizooties, Paris.
- Olivier G. (2002) Disease interactions between wild and cultured fish – perspectives from the American Northeast (Atlantic Provinces). *Bulletin of the European Association of Fish Pathologists* **22**, 103–109.
- Ross K., McCarthy U., Huntly P.J., Wood B.P., Stuart E., Rough E.I., Smail D.A. & Bruno D.W. (1994) An outbreak of viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*) in Scotland. *Bulletin of the European Association of Fish Pathologists* **14**, 213–214.
- Schlottfeldt H.J., Ahne W., Vestergaard Jørgensen P.E. & Glende W. (1991) Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) – a natural outbreak. *Bulletin of the European Association of Fish Pathologists* **11**, 105–107.
- Skall H.F., Olesen N.J. & Møllergaard S. (2005) Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review. *Journal of Fish Diseases* **28**, 509–529.
- Smail D.A. (1999) Viral haemorrhagic septicaemia. In: *Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections* (ed. by P.T.K. Woo & D.W. Bruno), pp. 123–147. CAB International, New York, NY.
- Smail D.A. (2000) Isolation and identification of viral haemorrhagic septicaemia (VHS) viruses from cod *Gadus morhua* with the ulcer syndrome and from haddock *Melanogrammus aeglefinus* having skin haemorrhages in the North Sea. *Diseases of Aquatic Organisms* **41**, 231–235.
- Snow M., Cunningham C.O., Melvin W.T. & Kurath G. (1999) Analysis of the nucleoprotein gene identifies distinct lineages of viral haemorrhagic septicaemia virus within the European marine environment. *Virus Research* **63**, 35–44.
- Snow M., Bain N., Black J., Taupin V., Cunningham C.O., King J.A., Skall H.F. & Raynard R.S. (2005) Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* **61**, 11–21.
- Stone D.M., Way K. & Dixon P.F. (1997) Nucleotide sequence of the glycoprotein gene of viral haemorrhagic septicaemia (VHS) viruses from different geographical areas: a link between VHS in farmed fish species and viruses isolated from North Sea cod (*Gadus morhua* L.). *Journal of General Virology* **78**, 1319–1326.
- Takano R., Nishizawa T., Arimoto M. & Muroga K. (2000) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild Japanese flounder, *Paralichthys olivaceus*. *Bulletin of the European Society of Fish Pathologists* **20**, 186–192.
- Takano R., Mori K., Nishizawa T., Arimoto M. & Muroga K. (2001) Isolation of viruses from wild Japanese flounder, *Paralichthys olivaceus*. *Fish Pathology* **36**, 153–160.
- Thiéry R., de Boissésion C., Jeffroy J., Castric J., de Kinkelin P. & Benmansour A. (2002) Phylogenetic analysis of viral

- haemorrhagic septicaemia virus (VHSV) isolates from France (1971–1999). *Diseases of Aquatic Organisms* **52**, 29–37.
- Winton J.R. & Einer-Jensen K. (2002) Molecular diagnosis of infectious hematopoietic necrosis and viral hemorrhagic septicemia. In: *Molecular Diagnosis of Salmonid Diseases* (ed. by C. Cunningham), pp. 49–79. Kluwer, Dordrecht.
- Wolf K. (1988) Viral hemorrhagic septicemia. In: *Fish Viruses and Fish Viral Diseases* (ed. by K. Wolf), pp. 217–248. Cornell University Press, Ithaca, NY.

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